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TITLE: Disparate Vitamin D Activity in the Prostate of Men with African Ancestry

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## **Introduction**

African American (AA) men are disproportionately affected by prostate cancer (PCa). AA men are not only at increased risk of PCa compared to American men of European descent (EA), but also are at the highest risk of aggressive PCa and death from PCa. Vitamin D3 deficiency increases PCa mortality, highlighting the importance of maintaining adequate vitamin D3 status for prostate health. Vitamin D3 is acquired in the diet or via UVB/sunlight-initiated synthesis in the skin. Cutaneous melanin absorbs UVB radiation, which leads to reduced vitamin D3 synthesis in darker pigmented skin. Consequently, ~65% of AA men are vitamin D3 deficient compared to ~20% of EA men. The level of skin pigmentation is correlated with the extent of African ancestry and serum vitamin D3 status. Besides vitamin D3 status, the activity of vitamin D3 is mediated by the vitamin D receptor (VDR) and determined by several cytochrome P450 metabolism enzymes that bioactivate/inactivate the active form of the hormone, 1,25-dihydroxyvitamin D3 (1,25D).

We hypothesized that the high prevalence of vitamin D3 deficiency in AA men is associated with reduced prostatic concentrations of vitamin D3, which leads to lower expression of vitamin D pathway genes and suppress pro-differentiating actions of vitamin D3 in the prostate; this ultimately abrogates the chemoprotective effects of this natural hormone and raises the susceptibility of AA men to aggressive PCa.

## **Keywords**

Vitamin D, prostate cancer, African-American

## Overall Project Summary

This research proposal brings together two well known health disparities that affect African-American men. The first is that African-American men are disproportionately affected by prostate cancer in that African-American men are not only at increased risk of prostate cancer compared to American men of European descent, but also are at the highest risk of aggressive prostate cancer and death from prostate cancer. The second disparity is the rampant vitamin D3 deficiency in the African-American population. There is a biological component to this deficiency because sun-induced vitamin D3 synthesis in the skin is significantly reduced in melanin-rich pigmented skin. Consequently, about two thirds of African-American men are vitamin D3 deficient compared to about 20% of men of European descent. It is important to maintain a healthy vitamin D3 status because vitamin D3 deficiency increases the risk of prostate cancer mortality.

Our study will directly examine the amount of vitamin D3 in the prostate tissue of a racially diverse group of patients to discern differences in African-American men. Secondly, we will investigate several mediators of vitamin D3 activity in the prostate tissue and in a novel cell culture model to identify innate molecular differences that may be present between men of European descent and in African-American men that increase susceptibility to aggressive prostate cancer in the latter group.

## Key Research Accomplishments

In year one of this award we have made significant progress in both the patient sample analyses and the *in vitro* assays.

With the patient samples, we completed African ancestry estimation and measurement of serum vitamin D metabolites in all of the patients for these study (**Figure 1**). *Of note, we changed cohorts as the patient specimens we planned to use (N=50 from collaborator Vince Freeman) became unavailable due to a freezer meltdown. We were able to acquire the necessary samples of frozen prostate tissue, serum and whole blood from 50 patients (25 AA and 25 EA) by collaborating with Dr. Peter Gann (here at UIC ) and via purchase from the Cooperative Human Tissue Network (CHTN).* In our cohort, the percentage of African Ancestry in men ranged from 2-95% (**Figure 1A**), which demonstrates the diverse ethnic background of self-declared black men and underscores the value of this additional analysis in interpreting our final data sets.

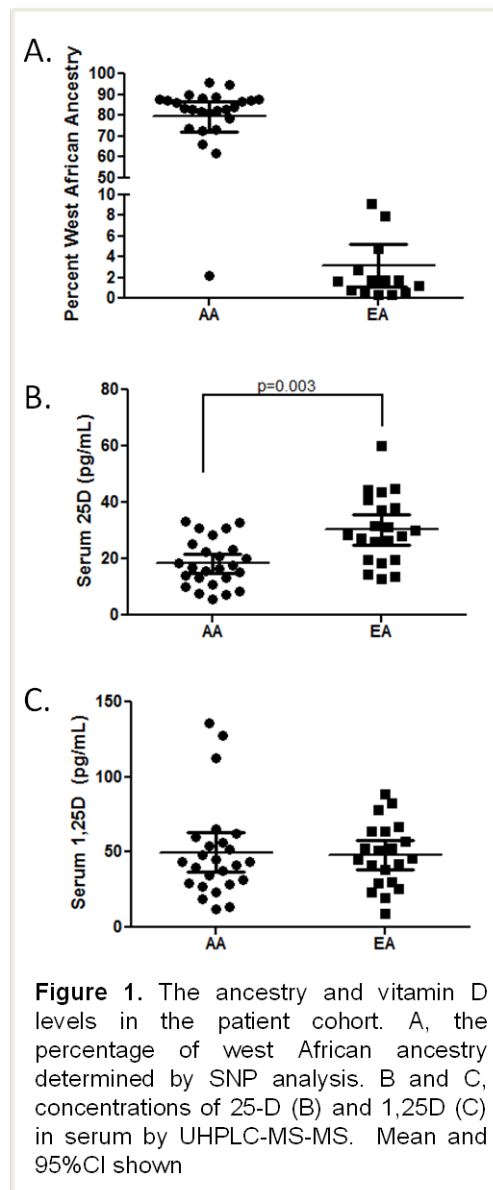
Serum measurement of 25-hydroxyvitamin D (25D) is used to determine vitamin D status and the AA patients had significantly lower 25D (**Figure 1B**). The levels of 1,25-dihydroxyvitamin D (1,25D), the active hormone, were also measured and not significantly different (**Figure 1C**).

The method for tissue extraction and measurement of the vitamin D metabolites has been fully optimized and we will complete the remaining tissues shortly. This technique proved to be more challenging than expected and Dr. van Breemen had difficulty measuring the vitamin D metabolites in the patient samples here at UIC. The vitamin D measurement was done in collaboration with Heartland Assays, a lab that only measures vitamin D metabolites and was started by the world renowned vitamin D expert Bruce Hollis. Heartland has quickly measured all of our serum samples and has begun measurement in the frozen human prostate tissues.

Laser-capture microdissection (LCM) on the frozen prostate tissues has been started and we expect to complete it within the next six months.

In the primary prostatic epithelial cells cultures we have begun to determine African ancestry and identify cells for the *in vitro* differentiation assays.

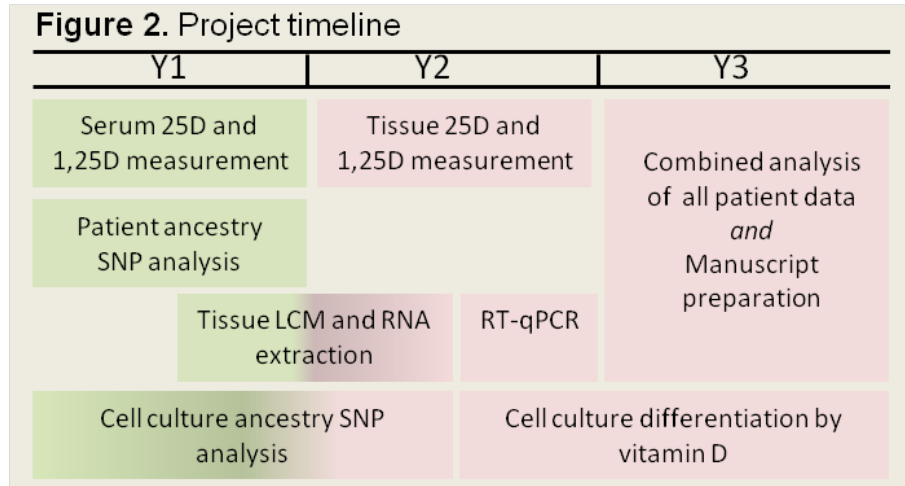
Dr. Kittles moved from UIC to the University of Arizona in September of 2014. The budget has been revised and submitted for a subcontract with the U of A. Dr. Kittles remains an active collaborator at 10% effort and we do not foresee any difficulty with continuing this collaborative project.



**Figure 1.** The ancestry and vitamin D levels in the patient cohort. A, the percentage of west African ancestry determined by SNP analysis. B and C, concentrations of 25-D (B) and 1,25D (C) in serum by UHPLC-MS-MS. Mean and 95%CI shown

## Conclusion

To date, we have completely analyzed serum vitamin D metabolites and African ancestry in a cohort of 50 diverse prostate cancer patients. Similar with other reports, vitamin D status in the AA patients is lower than the EA as measured by serum 25D. Molecular analysis of the patient samples has begun and we are slightly ahead of our anticipated schedule to complete Aims 1 and 3 of the project. Aim 2, which is *in vitro*, is also on schedule. Our proposed timeline for our current status and remainder of the project is shown in **Figure 2**.



## **Publications, Abstracts, and Presentations**

Publications: none

Abstract and Invited Presentation: Dec 2013 American Association for Cancer Research (AACR)- The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved, Atlanta, GA. “Vitamin D and prostate Cancer in African-American Men”

## **Inventions, Patents and Licenses**

none

## **Reportable Outcomes**

none

## **Other Achievements**

none

## **References**

none

## **Appendices**

none